The Involvement of Phosphatidate Phosphohydrolase and Phospholipase A Activities in the Control of Hepatic Glycerolipid Synthesis

EFFECTS OF ACUTE FEEDING WITH GLUCOSE, FRUCTOSE, SORBITOL, GLYCEROL AND ETHANOL

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Rats were fed by stomach tube with a single dose of glucose, sorbitol, fructose, glycerol or ethanol of equivalent energy contents or with 0.15m-NaCl. They were killed 6h later and the relative rates of phosphatidate deacylation and dephosphorylation measured in the microsomal and supernatant fractions of the livers. Treatment with sorbitol, fructose, glycerol and ethanol increased phosphohydrolase activities in the microsomal and supernatant fractions. The only significant change in deacylase activity was an increase in the microsomal fraction produced by ethanol. It is proposed that hepatic triacylglycerol synthesis is partly controlled by the balance between phosphatidate phosphohydrolase and phospholipase A-type activities.

The activity of the soluble phosphatidate phosphohydrolase (EC 3.1.3.4) in the liver responds rapidly and dramatically to changes in the physiological and pharmacological status of animals. The changes parallel the ability of the liver to synthesize triacylglycerols and the phosphohydrolase appears to have a major function in regulating this synthesis (Fallon et al., 1977; Brindley, 1978a,b). However, a restriction in the flux of phosphatidate to diacylglycerol ought to lead to the accumulation of phosphatidate, or to the synthesis of acidic phospholipids derived from CDP-diacylglycerol. The rates of synthesis of these lipids relative to those of triacylglycerols in the liver are low, and it therefore appears likely that excess phosphatidate is removed by deacylation. This sub- strate cycle of phosphatidate synthesis and degradation could be involved in the control of triacylglycerol synthesis (Whiting et al., 1977; Brindley, 1978a,b).

At the beginning of this work the relationship between the rate of phosphatidate deacylation and diacylglycerol synthesis was not known. It was, however, established that subcellular fractions of the liver could readily deacylate phosphatidate when it was added as an emulsion or as a membrane-bound substrate (Smith et al., 1967; Mitchell et al., 1971; Tzur & Shapiro, 1976; Sturton & Brindley, 1978).

In the present experiments rats were intubated with a single dose of saline, glucose, fructose, sorbitol, glycerol or ethanol. It was expected that the fructose (Fallon et al., 1977), sorbitol (Savolainen & Hassinen, 1977), glycerol (Savolainen & Hassinen, 1977) and ethanol (Lamb & Fallon, 1977; Savolainen, 1977; Pritchard et al., 1977) would produce large increases in the hepatic activities of the soluble phosphatidate phosphohydrolase, whereas the glucose and saline would not (Savolainen, 1977). This increase was then to be compared with the microsomal activity of phosphatidate phosphohydrolase and the rate of phosphatidate deacylation in the microsomal and soluble fractions derived from the livers. These activities can be measured simultaneously by using 1,2-diacyl-sn-[1,3-3H]glycerol 3-phosphate as a substrate (Sturton & Brindley, 1978).

Materials and Methods

The source or preparation of most of these has already been described (Pritchard et al., 1977; Whiting et al., 1977; Sturton & Brindley 1978).

Treatment of animals and preparation of subcellular fractions

Male Wistar rats (180-200g) were obtained from the Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K., and were allowed free access to a normal laboratory diet and to water before experiments. Rats were treated between 07:30 and 10:00h by stomach tube with a single dose of 20% (v/v) ethanol (5g/kg body wt) or with an equal volume of isocaloric glucose (9.5g/kg), fructose (9.5g/kg), glycerol (8.3g/kg) or sorbitol (9g/kg). Control animals were intubated with an equal volume of 0.15m-NaCl.
The rats were then deprived of food, but not of water. Then 6h after treatment the rats were killed by cervical dislocation and the livers perfused with ice-cold 0.15M-NaCl. Microsomal and particle-free supernatant fractions were prepared from liver homogenates (Mangiapanne et al., 1973). The microsomal recovery was determined using arylesterase (EC 3.1.1.2) as a marker for this fraction (Mangiapanne et al., 1973).

Measurement of the deacylation and dephosphorylation of phosphatidate

Each assay contained in 0.25ml: 100mm-Tris buffer, adjusted to pH7.4 with HCl, 1mm-dithiothreitol, 0.1mm-EGTA, 5mm-MgCl2, 2mg of fatty acid-poor bovine serum albumin/ml, 1.5mm-potassium 1,2-diacyl-sn-[[3H]gycerol 3-phosphate (0.5µCi/µmol) and either 50–300µg of soluble protein or 50–200µg of microsomal protein. Reactions were started with phosphatidate and incubated at 37°C for 20min for the soluble protein or 15min for the microsomal protein. The reactions were stopped by the addition of 1.88ml of chloroform/methanol (1:2, v/v) and the products were extracted by the method of Bligh & Dyer (1959), except that 0.1m-HCl was used instead of water to separate the phases.

The activity of phosphatidate phosphohydrolase was measured by the formation of 1,2-diacyl-sn-[[3H]glycerol. This was recovered from the bottom phase and purified on 5g columns of alkaline aluminium oxide using 15ml of chloroform/methanol (19:1, v/v) for elution. The deacylation of phosphatidate was measured by the appearance of watersoluble [3H]In 1.8ml samples of the top phase. The microsomal fraction yields a mixture of [3H]glycerol and [3H]glycerol phosphate, whereas the soluble fraction produces predominantly [3H]glycerol phosphate (Sturton & Brindley, 1978). The rates of formation of diacylglycerol and water-soluble products were proportional to the time of incubation and to the concentration of microsomal and soluble protein.

Determination of DNA, protein and radioactivity

DNA and protein were determined by the methods of Hübscher et al. (1965) and radioactivity was measured as described by Manning & Brindley (1972).

Results and Discussion

Intubating rats with a single dose of ethanol produced a mean increase of 4.3-fold in the soluble activity of phosphatidate phosphohydrolase when compared with rats intubated with glucose or saline (Table 1). The equivalent increases for sorbitol, fructose and glycerol were about 2.3-fold.

The microsomal activity of phosphatidate phosphohydrolase was increased by about 3.2-fold after intubation with ethanol compared with glucose (Table 1), although we reported no change in a previous study (Pritchard et al., 1977). In the latter work, this activity was measured at pH 6.4 rather than at 7.4, and by determining the release of P1. It is now known that this assay is unreliable for microsomal fractions since P1 is also formed via the deacylation of phosphatidate followed by the hydrolysis of glycerol phosphate (Sturton & Brindley, 1978). Lamb & Fallon (1977) have reported a 2-fold increase in the microsomal activity of phosphatidate phosphohydrolase 24h after ethanol administration, but the method of assay was not reported. Intubating rats with glycerol or sorbitol also increased the microsomal activity of phosphatidate phosphohydrolase by about 2-fold, but the increase after fructose

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Table I. Effect of treating rats with saline, glucose, sorbitol, fructose, glycerol and ethanol on the rates of deacylation and dephosphorylation of phosphatidate

Rats were intubated with a single dose of the various compounds and killed 6h later. Enzyme activities (means ± S.E.M.) are expressed as nmol of phosphatidate converted/min per µmol of DNA phosphorus. The total activity is the sum of the microsomal and soluble activities. The significance of the differences between the groups is shown by: *P< 0.05; †P< 0.005; ‡P< 0.001. The numbers of rats are shown in parentheses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Phosphatidate phosphohydrolase activity</th>
<th>Phospholipase A-type activity</th>
<th>Total phosphohydrolase</th>
<th>Total phospholipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Soluble</td>
<td>Microsomal</td>
<td>Total</td>
<td>Soluble</td>
</tr>
<tr>
<td>I</td>
<td>Saline (7)</td>
<td>62±8</td>
<td>72±10</td>
<td>135±15</td>
<td>17±3</td>
</tr>
<tr>
<td>II</td>
<td>Glucose (6)</td>
<td>61±3</td>
<td>61±7</td>
<td>121±11</td>
<td>19±4</td>
</tr>
<tr>
<td>III</td>
<td>Sorbitol (7)</td>
<td>143±15</td>
<td>123±12</td>
<td>266±21</td>
<td>22±4</td>
</tr>
<tr>
<td>IV</td>
<td>Fructose (6)</td>
<td>141±15</td>
<td>92±17</td>
<td>233±30</td>
<td>21±6</td>
</tr>
<tr>
<td>V</td>
<td>Glycerol (5)</td>
<td>154±74</td>
<td>123±9</td>
<td>274±34</td>
<td>19±4</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanol (7)</td>
<td>269±31</td>
<td>195±17</td>
<td>464±39</td>
<td>30±5</td>
</tr>
</tbody>
</table>

1978
administration was not significant (Table 1). However, it is known that feeding rats with diets rich in fructose for 60h results in a 3-fold increase in both the soluble and microsomal phosphohydrolase activities (Lamb & Fallon, 1974). When the results from the 38 rats described in Table 1 were subjected to linear-regression analysis there was a highly significant correlation ($r = 0.78; P < 0.001$) between the soluble and microsomal activities of phosphatidate phosphohydrolase.

The role of the microsomal phosphohydrolase in triacylglycerol synthesis has been difficult to assess, since (a) its activity may result from more than one enzyme (Jamdar & Fallon, 1973; Lamb & Fallon, 1974; Kako & Patterson, 1975; Caras & Shapiro, 1976) and (b) its activity may often have been over-estimated when it has been assayed by determining $P_i$ release (Sturton & Brindley, 1978). The results in Table 1 are not subject to the latter problem and they show that the microsomal activity is similar to that in the soluble fraction under the experimental conditions that were chosen. The two activities change rapidly and in parallel and both are probably involved in controlling triacylglycerol synthesis. It is not certain whether the same enzyme as exists in the soluble fraction is also found in the microsomal fraction. However, both activities are probably involved in triacylglycerol synthesis since maximum rates are only normally obtained when the soluble fraction is added back to the microsomal fraction (Hübscher et al., 1967).

There were significant correlations between the microsomal activities of the phosphohydrolase and deacylase ($r = 0.65; P < 0.001$), between the soluble activities of the phosphohydrolase and deacylase ($r = 0.42; P < 0.001$), but not between the deacylase activities in the microsomal and soluble fractions ($r = 0.044$). The only significant changes in deacylase rates were produced by ethanol, which increased the activities in the microsomal fraction and the combined activity of the microsomal and soluble fraction (Table 1). Under these experimental conditions, the rate at which phosphatidate was deacylated in the microsomal fraction was about 4 times that in the soluble fraction for all groups of rats.

The results in Table 1 are only for one time interval after the various treatments, since the purpose of the investigation was to study the relationship between the dephosphorylation and deacylation of phosphatidate. The effectiveness of the various treatments cannot be compared since changes in enzymic activities will probably depend on the rates of absorption and metabolism of the different compounds. It is, however, known that saline and glucose do not change the activity of the soluble phosphohydrolase at any time after intubation (Savolainen, 1977). The mechanism by which sorbitol, fructose, glycerol and ethanol increase this activity is not known. Savolainen & Hassinen (1977) suggested that this might be caused by the increased hepatic concentrations of glycerol phosphate. Feeding sorbitol, glycerol and ethanol should also change the redox state of the liver (Hawkins & Kalant, 1972), whereas feeding fructose would not (Pereira & Jangaard, 1971). However, doubts have been expressed that changes in glycerol phosphate concentrations alone can account for the ethanol-induced stimulation of triacylglycerol synthesis (Kalant et al., 1972; Fellenius et al., 1973; Estler, 1974; Abrams & Cooper, 1976). Also, the administration of benfluorex to rats before feeding ethanol partially prevented the increase in soluble phosphohydrolase activity (Pritchard et al., 1977), but it did not alter the rate of ethanol oxidation by the rats (Pritchard & Brindley, 1977).

The deacylation of phosphatidate could serve two functions in triacylglycerol synthesis. First, the cycle of acylation and deacylation might be required to produce diacylglycerol, which can be further acylated to triacylglycerol (Tzur & Shapiro, 1976). Feeding sorbitol, fructose, glycerol and ethanol markedly stimulated phosphohydrolase activity, but only ethanol increased the deacylase activity by about 1.5-fold (Table 1). This may mean (a) that the deacylase is not directly involved in promoting triacylglycerol synthesis, (b) that its activity may already be adequate to cope with large increases in this synthesis or (c) that a large increase in a specific phosphatidate deacylase is masked by non-specific phospholipase A activities. The alternative function of the deacylase may be to remove excess phosphatidate from the endoplasmic-reticulum membranes (Tzur & Shapiro, 1976; Whiting et al., 1977). The deacylase activity does not behave in a reciprocal manner to that of the phosphohydrolase in the present experiments. However, the balance between deacylation and dephosphorylation is increased in favour of diacylglycerol synthesis by about 1.8–2.0-fold after feeding sorbitol, fructose and glycerol and by 2.5-fold with ethanol (Table 1). These results are compatible with the hypothesis (Brindley, 1978a,b) that the rate of hepatic triacylglycerol synthesis is partly controlled by the rate at which phosphatidate is converted into diacylglycerol or recycled back to glycerol phosphate.

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References

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